



Draft Genome Sequences of 12 Clinical and Environmental Methicillin-Resistant *Staphylococcus pseudintermedius* Strains Isolated from a Veterinary Teaching Hospital in Washington State

Devendra H. Shah,^a Lisa P. Jones,^b Narayan Paul,^a  Margaret A. Davis^b

^aDepartment of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington, USA

^bPaul G. Allen School for Global Animal Health, Washington State University, Pullman, Washington, USA

ABSTRACT Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a globally emergent multidrug-resistant pathogen of dogs associated with nosocomial transmission in dogs and with potential zoonotic impacts. Here, we report the draft whole-genome sequences of 12 hospital-associated MRSP strains and their resistance genotypes and phenotypes.

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is an opportunistic canine pathogen that causes infections of the skin and soft tissue, such as superficial or deep pyoderma, wounds, urinary tract, and other body sites, including otitis media or externa, and is often associated with surgical site infections (1, 2). MRSP is also commonly isolated from cats (3–5) and occasionally from rats, cows, and horses (6–8). Hospitalization and antimicrobial treatment are major risk factors for MRSP colonization or infection, making this an important pathogen associated with veterinary hospital-acquired infections (9–16). The number of MRSP/total *S. pseudintermedius* infections in hospitalized animals at the Washington State University Veterinary Teaching Hospital increased from 1/17 in 2009 to 11/46 in 2017, peaking at 22/71 in 2015; this increase is consistent with recent increases reported worldwide (1). The potential for zoonotic transmission to humans (11, 17–20), multidrug resistance (MDR), and concern that MRSP could be mistaken for methicillin-resistant *Staphylococcus aureus* (MRSA) (20, 21) suggest the need for improved detection and molecular subtyping tools for epidemiological source tracing of this pathogen, especially during outbreaks. Here, we report draft genome sequences of 12 MDR-MRSP clinical and environmental strains isolated from a veterinary teaching hospital in Washington State (Table 1). A single colony of each MDR-MRSP strain was grown overnight at 37°C in brain heart infusion (BHI) broth (Difco). DNA was extracted using zirconia beads and the Qiagen DNeasy tissue kit (Qiagen, USA), with the exception that additional 70% ethanol washes and RNase incubations were added. Paired-end sequencing libraries (2 × 150 bp) were prepared using NEBNext Ultra II kit (NEB, UK), according to the manufacturer's protocol, and size selected in the range of 422 to 502 bp (average size, ~458 bp). All strains were sequenced using the Illumina HiSeq 4000 platform (Illumina, Inc., USA). Sequences were trimmed using BBDuk and *de novo* assembled using Velvet 1.2.10, with the k-mer length set at 99 (22). Contigs were reorganized by aligning to the genome sequence of the reference *S. pseudintermedius* strain 081661 (GenBank accession no. NZCP016073) (23) using progressiveMauve (24). Automated annotation of the assembled contigs was performed using the NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Whole-genome multilocus sequence typing

Received 7 March 2018 Accepted 17 March 2018 Published 12 April 2018

Citation Shah DH, Jones LP, Paul N, Davis MA. 2018. Draft genome sequences of 12 clinical and environmental methicillin-resistant *Staphylococcus pseudintermedius* strains isolated from a veterinary teaching hospital in Washington State. *Genome Announc* 6: e00290-18. <https://doi.org/10.1128/genomeA.00290-18>.

Copyright © 2018 Shah et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Margaret A. Davis, madavis@wsu.edu.

TABLE 1 GenBank accession numbers for 12 *Staphylococcus pseudintermedius* strains

Strain	WGS accession no. ^a	No. of paired reads	No. of contigs	N ₅₀ (bp)	Genome length (bp)	G+C content (%)	Source	Yr	Resistance profile ^b	Resistance genes	MLST ^c
200	PHHV000000000	10,091,826	38	134,914	2,620,679	37.4	Skin swab	2011	Ak Aug Am Cfx Vec Cfx Cpd Xnl Cd E Gm Imp Ox P Ti Tim Sxt	<i>aac(6)-aph(2'')</i> , <i>ant(6)-la</i> , <i>aph(3)-III</i> , <i>mecA</i> , <i>blaZ</i> , <i>erm(B)</i> , and <i>dfg</i>	64
335	PHHW000000000	10,437,560	51	133,420	2,710,949	37.4	Leg edema	2012	Aug Am Cef Vec Cfx Cpd Xnl Cli Eno E Imp Mar Oxa P Ti Tim Sxt	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>mecA</i> , <i>blaZ</i> , <i>erm(B)</i> , and <i>dfg</i>	71
473	PHHX000000000	10,309,238	42	133,290	2,744,531	37.3	Thoracic fluid	2013	Ak Aug Am Cfx Vec Cpd Xnl Cd Eno E Gm Imp Mar Ox Tim Ti Sxt	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , and <i>dfg</i>	71
476	PHID000000000	8,007,918	41	133,420	2,745,474	37.3	Thoracic fluid	2013	Ak Aug Am Cfx Vec Cpd Xnl Cd Eno E Gm Imp Mar Ox Tim Ti Sxt	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , and <i>dfg</i>	71
586	PHIC000000000	7,874,460	42	151,204	2,665,706	37.4	Urine	2014	Aug Am Cfx Vec Cfx Cpd Xnl Cli Eno E Mar Oxa P Ti Tim Sxt	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , and <i>dfg</i>	71
651	PHIB000000000	9,350,720	37	133,419	2,698,461	37.4	Clippers	2015	Ak Aug Am Cfx Vec Cfx Cpd Xnl Cd Eno E Gm Mar Ox P Ti Tim Sxt	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , and <i>dfg</i>	71
684	PHIA000000000	7,436,180	49	120,128	2,668,380	37.4	Surgical plate	2015	Ak Aug Am Cfx Vec Cfx Cpd Xnl C Cd Eno E Gm Mar Ox P Ti Tim Sxt	<i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , and <i>erm(B)</i>	84
738	PHHZ000000000	9,172,346	38	171,264	2,640,001	37.4	Clippers	2015	Dox Eno E Gm Mar Ox P Ti Tim Sxt C Cd Eno E Gm Mar Ox P Ti	<i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>aac(6)-aph(2'')</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , <i>cat(pC221)</i> , and <i>tet(M)</i>	45
742	PHHY000000000	10,507,688	37	171,307	2,637,262	37.4	Nasal swab	2015	Ak Aug Am Cfx Vec Cfx Cpd Xnl C Cd Eno E Gm Mar Ox P Ti Tim Ti	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , <i>cat(pC221)</i> , and <i>tet(M)</i>	45
424	PRDQ000000000	8,440,642	43	134,451	2,660,157	37.3	Swab	2012	Ak Aug Am Cfx Vec Cfx Cpd Cd Eno E Gm Imp Ti Tim Sxt	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>erm(B)</i> , <i>tet(M)</i> , and <i>dfg</i>	924
980	PRDR000000000	7,516,686	45	156,632	2,634,738	37.4	Clippers	2017	Aug Am Cfx Vec Cpd Cf Cd Dox Eno E Gm Min Ox P Te	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , and <i>tet(M)</i>	45
1019	PRDP000000000	7,956,734	46	133,401	2,737,904	37.2	Abscess fluid	2017	Aug Am Cfx Cpd Cf C Cd Dox Eno E Mar Te Sxt	<i>aac(6)-aph(2'')</i> , <i>aph(3)-III</i> , <i>ant(6)-la</i> , <i>blaZ</i> , <i>mecA</i> , <i>erm(B)</i> , <i>cat(pC221)</i> , <i>tet(M)</i> , and <i>dfg</i>	930

^aWGS, whole-genome sequencing.

^bAntibiotic abbreviations: Ak, amikacin; Am, ampicillin; Aug, amoxicillin-clavulanic acid; C, chloramphenicol; Cd, clindamycin; Cf, cefazolin; Cfx, cefoxitin; Cfd, cefepodoxime; Dox, doxycycline; E, erythromycin; Eno, enrofloxacin; Gm, gentamicin; Imp, imipenem; Mar, marbofloxacin; Min, minocycline; Ox, oxacillin; P, penicillin; Ti, ticarcillin; Tim, ticarcillin-clavulanic acid; Sxt, trimethoprim-sulfamethoxazole; Vec, cefovecin; Xnl, ceftiofur.

^cMLST, multilocus sequence type.

was performed using the *Staphylococcus pseudintermedius* MLST database (<https://pubmlst.org/spseudintermedius/>), as described previously (25). All of these strains displayed multidrug resistance (resistance to >3 classes of antibiotics) when MICs were measured according to current Clinical and Laboratory Standards Institute (CLSI) protocols (26). The corresponding resistance genes were identified using ResFinder version 3.0 (27). All strains contained staphylococcal cassette chromosome *mec* elements (SCC*mec*), as identified by ResFinder version 3.0. These genome sequences will aid in the development of improved molecular diagnostics and subtyping methods for epidemiological source tracing of MRSP outbreaks. The detailed comparative genomics analysis of these strains is currently ongoing and will be published independently.

Accession number(s). The sequences have been deposited in the NCBI GenBank database. The accession numbers are listed in Table 1.

ACKNOWLEDGMENT

This project is funded by the Morris Animal Foundation grant D17CA-019, "Evaluation of genotyping methods for *Staphylococcus pseudintermedius* at local, regional and global scales."

REFERENCES

- van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyörälä S, Ruzauskas M, Sanders P, Threlfall EJ, Torren-Edo J, Törneke K, Scientific Advisory Group on Antimicrobials (SAGAM). 2011. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother 66: 2705–2714. <https://doi.org/10.1093/jac/dkr367>.
- Nazarali A, Singh A, Moens NMM, Gatineau M, Sereda C, Fowler D, Kim SE, Kisiel A, Reynolds D, Ringwood BR, Bruce CW, Gibson TWG, Rousseau J, Weese JS. 2015. Association between methicillin-resistant *Staphylococcus pseudintermedius* carriage and the development of surgical site infections following tibial plateau leveling osteotomy in dogs. J Am Vet Med Assoc 247:909–916. <https://doi.org/10.2460/javma.247.8.909>.
- Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach G-F, Schwarz S, Kreienbrock L, Nolte I, Simon D. 2011. Methicillin-resistant *Staphylococcus pseudintermedius* among cats admitted to a veterinary teaching hospital. Vet Microbiol 153:414–416. <https://doi.org/10.1016/j.vetmic.2011.05.045>.
- Kadlec K, Weiß S, Wendlandt S, Schwarz S, Tonpitak W. 2016. Characterization of canine and feline methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from Thailand. Vet Microbiol 194:93–97. <https://doi.org/10.1016/j.vetmic.2016.04.015>.
- Kadlec K, Schwarz S, Perreten V, Andersson UG, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L. 2010. Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. J Antimicrob Chemother 65: 1826–1828. <https://doi.org/10.1093/jac/dkq203>.
- Himsworth CG, Patrick DM, Parsons K, Feng A, Weese JS. 2013. Methicillin-resistant *Staphylococcus pseudintermedius* in rats. Emerg Infect Dis 19:169–170. <https://doi.org/10.3201/eid1901.120897>.
- Pilla R, Bonura C, Malvisi M, Snel GGM, Piccinini R. 2013. Methicillin-resistant *Staphylococcus pseudintermedius* as causative agent of dairy cow mastitis. Vet Rec 173:19.2. <https://doi.org/10.1136/vr.101485>.
- Gómez-Sanz E, Simón C, Ortega C, Gómez P, Lozano C, Zarazaga M, Torres C. 2014. First detection of methicillin-resistant *Staphylococcus aureus* ST398 and *Staphylococcus pseudintermedius* ST68 from hospitalized equines in Spain. Zoonoses Public Health 61:192–201. <https://doi.org/10.1111/zph.12059>.
- Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. 2007. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol 45:1118–1125. <https://doi.org/10.1128/JCM.02193-06>.
- Lehner G, Linek M, Bond R, Lloyd DH, Prenger-Berninghoff E, Thom N, Straube I, Verheyen K, Loeffler A. 2014. Case–control risk factor study of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) infection in dogs and cats in Germany. Vet Microbiol 168:154–160. <https://doi.org/10.1016/j.vetmic.2013.10.023>.
- van Duijkeren E, Houwers DJ, Schoormans A, Broekhuizen-Stins MJ, Ikwaty R, Fluit AC, Wagenaar JA. 2008. Transmission of methicillin-resistant *Staphylococcus intermedium* between humans and animals. Vet Microbiol 128:213–215. <https://doi.org/10.1016/j.vetmic.2007.11.016>.
- Grönthal T, Moodley A, Nykäsenoja S, Junnila J, Guardabassi L, Thomson K, Rantala M. 2014. Large outbreak caused by methicillin resistant *Staphylococcus pseudintermedius* ST71 in a Finnish veterinary teaching hospital—from outbreak control to outbreak prevention. PLoS One 9:e110084. <https://doi.org/10.1371/journal.pone.0110084>.
- Detwiler A, Bloom P, Petersen A, Rosser EJ. 2013. Multi-drug and methicillin resistance of staphylococci from canine patients at a veterinary teaching hospital (2006–2011). Vet Q 33:60–67. <https://doi.org/10.1080/01652176.2013.799792>.
- Ishihara K, Shimokubo N, Sakagami A, Ueno H, Muramatsu Y, Kadosawa T, Yanagisawa C, Hanaki H, Nakajima C, Suzuki Y, Tamura Y. 2010. Occurrence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* in an academic veterinary hospital. Appl Environ Microbiol 76: 5165–5174. <https://doi.org/10.1128/AEM.02780-09>.
- Kasai T, Saegusa S, Shirai M, Murakami M, Kato Y. 2016. New categories designated as healthcare-associated and community-associated methicillin-resistant *Staphylococcus pseudintermedius* in dogs. Microbiol Immunol 60:540–551. <https://doi.org/10.1111/1348-0421.12401>.
- Walther B, Tedin K, Lübke-Becker A. 2017. Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet Microbiol 200:71–78. <https://doi.org/10.1016/j.vetmic.2016.05.017>.
- Campanile F, Bongiorno D, Borbone S, Venditti M, Giannella M, Franchi C, Stefani S. 2007. Characterization of a variant of the SCC*mec* element in a bloodstream isolate of *Staphylococcus intermedium*. Microb Drug Resist 13:7–10. <https://doi.org/10.1089/mdr.2006.9991>.
- Somayaji R, Priyantha MAR, Rubin JE, Church D. 2016. Human infections due to *Staphylococcus pseudintermedius*, an emerging zoonosis of canine origin: report of 24 cases. Diagn Microbiol Infect Dis 85:471–476. <https://doi.org/10.1016/j.diagmicrobio.2016.05.008>.
- Stegmann R, Burnens A, Maranta CA, Perreten V. 2010. Human infection associated with methicillin-resistant *Staphylococcus pseudintermedius* ST71. J Antimicrob Chemother 65:2047–2048. <https://doi.org/10.1093/jac/dkq241>.
- Paul NC, Moodley A, Ghibaudo G, Guardabassi L. 2011. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in small animal veterinarians: indirect evidence of zoonotic transmission. Zoonoses Public Health 58:533–539. <https://doi.org/10.1111/j.1863-2378.2011.01398.x>.
- Viau R, Hujer AM, Hujer KM, Bonomo RA, Jump RL. 2015. Are *Staphylococcus intermedium* infections in humans cases of mistaken identity? A case series and literature review. Open Forum Infect Dis 2:ofv110. <https://doi.org/10.1093/ofid/ofv110>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. <https://doi.org/10.1101/gr.074492.107>.

23. Riley MC, Perreten V, Bemis DA, Kania SA. 2016. Complete genome sequences of three important methicillin-resistant clinical isolates of *Staphylococcus pseudintermedius*. *Genome Announc* 4:e01194-16. <https://doi.org/10.1128/genomeA.01194-16>.
24. Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res* 14:1394–1403. <https://doi.org/10.1101/gr.2289704>.
25. Solyman SM, Black CC, Duim B, Perreten V, van Duijkeren E, Wagenaar JA, Eberlein LC, Sadeghi LN, Videla R, Bemis DA, Kania SA. 2013. Multilocus sequence typing for characterization of *Staphylococcus pseudintermedius*. *J Clin Microbiol* 51:306–310. <https://doi.org/10.1128/JCM.02421-12>.
26. Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, 4th ed. CLSI document VET01-A4. Clinical and Laboratory Standards Institute, Wayne, PA.
27. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.